FIGURE 3 is an isometric view of the imaging apparatus in FIGURE 2A, showing different locations for one or more light source(s);

FIGURE 4 is an alternative embodiment of the imaging apparatus in FIGURES 2A and 3, in which a second set of imaging components and time delay integration (TDI) detector are included for monitoring light from a bead, to avoid interference between different reporters, and showing alternative locations for one or more light source(s);

FIGURE 5 is a schematic diagram illustrating the optical convolution of a narrow fluorescence *in situ* hybridization (FISH) emission spectrum by the present invention, to resolve two FISH probes in a cell;

FIGURE 6 is a schematic diagram showing the optical convolution of two different colors of narrow FISH emission spectra, to resolve the image of the FISH probes on the TDI detector;

FIGURE 7 is a schematic diagram illustrating how for a wider FISH emission spectrum, a deconvolution is provided to resolve the image of two FISH probes of a single color;

FIGURE 8 is a schematic diagram showing the deconvolution of two color FISH spectra that are relatively wide, to resolve the image of the FISH probes;

FIGURE 9 is a schematic block diagram of the system used to process the signal produced by a TDI detector in the present invention;

FIGURE 10 is a schematic diagram illustrating how an imaging system in accord with the present invention is used to determine whether a cell is from a male or female;

FIGURE 11 is a plan view of an alternate embodiment for an imaging system usable in the present invention that employs a spectral dispersion component comprising a plurality of stacked dichroic filters that spectrally separate the light;

FIGURE 12 is an X-Y plot of several typical passbands for the dichroic filters employed in the embodiment shown in FIGURE 11;

FIGURE 13 is a schematic illustration of a detection filter assembly that may optionally be placed in front of the TDI detector in the embodiment of FIGURE 11 to further suppress out-of-band light;

FIGURES 14A-14E are X-Y plots of transmission vs. wavelength corresponding to corresponding passbands of the filter segments of the detection filter assembly that may optionally be placed in front of the TDI detector;

FIGURE 15 is a plan view of another embodiment of the configuration of FIGURE 11, wherein the spectral dispersion filter system comprises a plurality of dichroic cube filters orientated at various angles to create the spectral dispersing effect;

FIGURE 16A illustrates another embodiment of the imaging system in accord with the present invention, in which the spectral emission is not convolved with the image and in which the spectral decomposition occurs in an axis perpendicular to flow through the use of separate dichroic filters, imaging lenses, and detectors for each spectral region;

FIGURE 16B illustrates yet another embodiment of the imaging system in accord with the present invention, in which the spectral emission is not convolved with the image and in which the spectral decomposition occurs in an axis perpendicular to flow through the use of one imaging lens and separate dichroic filters, and detectors for each spectral region;

FIGURE 16C is an isometric illustration of correction plates added to correct for astigmatism induced by a plate beam splitter placed in convergent space;

FIGURE 17 illustrates images that are projected onto a detector for the spectral decomposition embodiment when three beads are in view;

FIGURE 18A is a flow chart illustrating the steps employed in the method of the present invention for reading reporter labeled beads in a flow;

FIGURE 18B is a flow chart illustrating the steps employed in the method of the present invention for decoding the reporters on a reporter labeled bead;

FIGURE 19 is a reporter legend that aids in the identification of reporter labeled beads;

FIGURE 20 illustrates hybridizing oligos having sequences that are offset by one nucleotide, forming a contiguous overlapping string of sequences;

FIGURE 21 illustrates the application of the flow imaging sequencing method to determine the identity of SNPs;

FIGURE 22 is a flow chart illustrating the steps employed in the method of the present invention for constructing sequence contigs for identifying a DNA sequence, polymorphic alleles, or expressed genes;

FIGURE 23 is a plot of channel spectral passbands superimposed over emission spectra for common fluorochromes used in reporter beads, carriers, or binding signals;

FIGURE 24 is a block diagram illustrating the general steps employed in the method of the present invention for applying crosstalk corrections to reporter labeled bead imagery prior to decoding the reporter labeled bead assemblies;

FIGURE 25 is a block diagram for the steps of applying spectral and spatial corrections to reporter labeled bead imagery;

FIGURE 26 is a block diagram of the steps of generating the accumulated average correlograms between the data images and the reference; and

FIGURE 27 is an example of imagery before and after spectral and spatial correction.

Description of the Preferred Embodiment

It is contemplated that the present invention may be applied to combinatorially created beads and compounds as well as specifically directed synthesis of beads and compounds. Further details regarding both of these aspects of the present invention are discussed below.

New Method for Analyzing Reporter labeled Beads

A new bead imaging system and method for analyzing reporter labeled beads addresses the problems for carrying out this task that would arise in reading beads using any conventional approach and adds new capabilities to the analysis and handling of reporter labeled beads. The imaging system enables the discrimination of the different reporters attached to each bead and therefore enables the decoding of the complete reporter signature and corresponding chemical identity (e.g., oligonucleotide sequence) bound to the bead. By preferably handling beads in suspension and using hydrodynamic focussing, billion-count bead samples can easily be moved through the field of view (FOV) of a flow imaging system at high rates. Existing non-imaging flow cytometers cannot be used with this combinatorial scheme because on a given bead, different reporters with any colors in common at the same intensity cannot be distinguished from each other without spatial information. Similarly, without image information, different reporter sizes or shapes, which can confer part of the information included in a reporter signature, cannot be discriminated. It should be noted that while a preferred embodiment of the present invention contemplates imaging encoded beads while entrained in a flow of fluid, encoded beads can be imaged in stasis, and a flow is not required. It is contemplated that imaging encoded beads in a flow of fluid will enable a large number of beads to be rapidly imaged and analyzed. It is anticipated that the resolution afforded by the present invention will enable the reporters themselves to be small beads of polystyrene, silica, resin, or any another substance that can be fabricated or treated to possess a unique optical signature, which are then associated with larger (yet still relatively small) substrate beads.

An embodiment of the present invention for analyzing beads in flow includes subsystems for implementing the tasks of: (1) optical signal collection and spectral decomposition; (2) pixelated detection; (3) illumination; (4) bead velocity detection; and (5) sample handling. One or more embodiments of each of these subsystems is described in detail below.

An important aspect of the present invention lies in its ability to simultaneously discriminate the location of the various fluorescent emission spectra produced by